PAPER # 10

REISSUE **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of Beelman et al.

Reissue Serial No.: 09/899,090 Examiner: Tierney, Christine

Filed: July 5, 2001 Art Unit: 1700 (Special Programs)

U.S. Patent No. 5,919,507

For: **PRESERVATION COMPOSITIONS AND METHODS FOR MUSHROOMS**

Via Facsimile (703) 872-9743

Attn: Examiner Christine Tierney
Commissioner for Patents
Alexandria, VA 22313-1450

REQUEST FOR CONSIDERATION DURING REISSUE

Dear Commissioner:

The issue of whether the applicant in this reissue has actually disclosed and enabled its broadened reissue claims under the requirements of 35 USC §112 does not appear to have been fully considered by the USPTO.[1] In filing the reissue request, the patentee added 29 new claims, the broadest of which, claim 9, exceeds the scope of any of the originally issued claims. Other claims also exceed the scope of original claims.

[1] This conclusion is made based on the record available to the undersigned. The entire record was last checked at the end of February, 2003. During a recent attempt to copy the most recent documents of the record, the undersigned was denied access to the files because of their current status (being reviewed in the Special Programs Group of Technology Center 1700). Accordingly, the undersigned cannot determine the current wording of the claims actually under consideration.

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Claim 9 was presented as follows:

> 9. A method for preserving fresh and processed mushrooms comprising the following steps of:
>
> contacting the mushrooms briefly with an antimicrobial buffer solution having a pH of at least about 9; and
>
> rinsing the mushrooms one or more times after said contacting step with a pH-neutralizing solution having a sufficient pH to return the mushrooms to the mushroom physiological pH or about 6.5.[2]

In the original reissue request, the patentee stated that the broadened reissue claims were disclosed and supported and provided a document purporting to identify support for the added and broadened claims.[3] However, careful examination of the specification as a whole and of the "support section" identified by the patentee will show that at least the broadest of the new claims (i.e., those that attempt to eliminate use of erythorbate buffers in the wash step immediately after the high pH step) are in fact neither disclosed nor enabled.

FACTS RELEVANT TO REISSUE FILING

This reissue filing and the broadening of the claims were apparently caused by the filing of another patent application at the USPTO after the '507 patent was granted. That other application has now issued as U.S. Patent No. 6,500,476 to Martin et al. (the "'476 patent") and is assigned to EPL Technologies, Inc. ("EPL") of Philadelphia, PA. A copy of the EPL '476 patent is enclosed as Exhibit A. The '476 patent discloses a three-step wash method for cleaning and preserving mushrooms.

EPL was apparently working with Pennsylvania State University ("Penn State;" the assignee of the '507 patent) during the preparation and prosecution of the application leading to the '507 patent. As evidence of this relationship, a copy of a letter from Matthew Smith of the licensing office of Penn State to a potential licensee is enclosed (redacted to avoid identifying the potential licensee) as Exhibit B. This letter states that

[2] '507 patent reissue file history; original request for broadened claims.

[3] '507 patent reissue file history; Combined Reissue Application Declaration of the Inventor and Power of Attorney, p.1, and Statement of Claim Support and Status.

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the claims of the '507 patent were drafted by "EPL's counsel" (second paragraph of Exhibit B). This relationship is evidence of the working relationship between the two parties and supports the understanding of the undersigned that Penn State knew about the filing of the EPL patent and that such filing triggered this reissue. There is additional evidence of this triggering event, which can be provided to the USPTO should it consider the triggering event relevant to the issue described herein.

For the record, it should be noted that the undersigned does not represent either Penn State or EPL.

After the grant of the Penn State '507 patent, EPL appears to have realized that the '507 patent process is not particularly valuable from a commercial perspective. EPL then developed a commercially viable three-stage washing process that was not disclosed or claimed in the '507 patent. EPL's recognition of the failure of the '507 patent to describe a commercially viable process is highlighted in the text of the granted EPL '476 patent:

> In 1999, Beelman and Duncan incorporated the use of a high pH wash with an antibrowning solution in a two-stage process (U.S. Pat. No. 5,919,507). The Beelman-Duncan process used a high pH first stage as the antimicrobial treatment and a second stage of sodium erythorbate, calcium and EDTA to minimize enzymatic browning. *While this process combined and improved the teaching of McConnell and Sapers, it narrowly focused on a two-stage sequence with limited chemical selection in each process step. Specifically, the Beelman-Duncan process limited the pH neutralizing step to include solutions of erythorbic acid and sodium erythorbate. In addition, Beelman applied the erythorbic solution immediately after the antimicrobial contacting step. This restrictive sequence resulted in the rapid degradation of the sodium erythorbate solution in the neutralization stage and the need for increased quantities of the anti-browning materials. Consequently, the Beelman process did not address the variability in raw material and proved too expensive to be adopted by the processors and did not achieve commercial viability.*[4]

It is to "cure" the original claims of the Beelman (Penn State) '507 patent that the current reissue was apparently filed. This is evident from the reissue declaration, which contains the following statement:

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[4] '476 patent, col. 2, lines 33-51.

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> Applicants' attorney failed to appreciate the full scope of applicants' invention. The invention set forth in claim 1, the sole independent claim in the patent, unduly limits the pH-neutralizing solution used in the second stage washing step(s) to a preferred embodiment of buffer solutions of erythorbic acid and sodium erythorbate. The invention described and enabled in the specification is not limited to the used of erythorbic acid/sodium erythorbate buffer solutions to return the final mushroom pH to the physiological range of approximately 6.5. As described in the specification, acidulants and even water alone were used to neutralize the effects of exposure to high pH antimicrobial solutions in the first stage.[5]

While the undersigned agrees that part of this statement is true (i.e., the original granted claims are limited to use of washing steps containing an erythorbate buffer to reduce the high pH first step used to kill bacteria), other parts are inaccurate (and might even be considered misleading). Various inaccuracies are discussed below. However, they can be summarized as follows: *All* of the wash steps actually shown in the '507 specification and identified as producing *useful* results (either as specific processes or through generic language) *use erythorbate buffer in the wash steps immediately after the initial high pH step.* No buffer or acidulant other than an erythorbate buffer is ever described in the specification for use in the wash step after high pH treatment, and the only other wash that is described – water in one comparative example – is described as producing unsatisfactory results.

LACK OF DISCLOSURE AND ENABLEMENT OF BROADENED REISSUE CLAIMS

What the patentee in the '507 patent is attempting to do now, at least by the originally submitted broadened claims, is to eliminate the need to use an erythorbate buffer in the wash step(s) immediately after the initial high pH wash that is used to kill bacteria. It is attempting to claim *any* process used to reduce the pH in a second step (see claim 9, above). However, the '507 specification teaches no buffered wash solution or

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[5] Page 1 of Combined Reissue Declaration by the Inventor and Power of Attorney in current reissue file history. Note reference here to "Applicants' attorney" in the same context that Exhibit B (above) refers to "EPL's counsel."

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acidulant other than erythorbic acid/sodium erythorbate solutions and only uses water in one comparative example said to produce unsatisfactory results.

In that comparative example, water, without any additional buffering, was used, but the results were not considered to be satisfactory. In the second paragraph of the "Conclusions" recited at the end of the patent specification (just before the tables that precede the claims), the specification says that "*the erythorbic acid concentration could be reduced to as low as 0.4% and sodium erythorbate concentration as low as 1.6% (retaining the 1:4 erythorbic acid: sodium erythorbate ratio) in the second-stage wash.*"[6] There is no indication that the erythorbate buffer can be *eliminated* in the second wash step, which the patentee is now attempting to do in this reissue. This quoted selection from the conclusions is in a paragraph specifically directed to effective treatments: "*The treatment* was found to be robust, however, and *was effective over a range* of temperatures, holding times, and even wash solution ingredient *concentrations*."[7] There is no indication in the specification that the "treatment" was "effective" (i.e., useful) for different wash solution *ingredients*, as the broadened reissue claim are attempting to encompass.

For convenience, the "Conclusions" section of the specification is reproduced here in its entirety.

> CONCLUSIONS[8]
>
> A two-stage wash treatment consisting of a 0.05M sodium bicarbonate buffer at pH 10.5-11.0 in the first stage, followed by a neutralization solution containing 0.6% erythorbic acid, 2.4% sodium erythorbate, 1000 ppm EDTA, and 1000 ppm calcium chloride in the second stage is very effective at improving shelf life and quality of fresh and processed white mushrooms (Agaricus bisporus). This treatment equals the initial whiteness achieved by sulfite treatment, while controlling bacterial growth, preventing blotch and lesion formation, and improving shelf life and storage quality as effectively as or better than wash treatments incorporating hydrogen peroxide and EDTA.

[6] '507 patent; col. 18, lines 36-41.

[7] '507 patent; col. 18, lines 30-33.

[8] '507 patent; col. 18, lines 10-67.

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Wash solution temperatures and mushroom holding times in wash solutions affect the performance of the high-pH/erythorbate treatment. A retention time of 30 seconds in a pH 10.5-11.0 first-stage buffer at 25.degree. C., followed by 60 seconds in a 3% erythorbate solution at 10.degree. C. were determined to be optimal processing conditions. *The treatment was found to be robust, however, and was effective over a range* of temperatures, holding times, and even wash solution ingredient *concentrations*. The pH of the first-stage wash solution could be reduced to 9.5-10.0 without serious detriment to performance, particularly if the buffering capacity (sodium bicarbonate concentration) is increased. *Similarly, the erythorbic acid concentration could be reduced to as low as 0.4% and sodium erythorbate concentration as low as 1.6% (retaining the 1:4 erythorbic acid: sodium erythorbate ratio) in the second-stage wash.*

The addition of 1000 ppm EDTA and 1000 ppm calcium chloride to the second-stage wash solution enhanced the performance of the treatment, with each ingredient resulting in an improvement in mushroom color. EDTA functions to chelate copper, a cofactor of polyphenol oxidase, the browning enzyme in mushrooms. It has also been shown to enhance the performance of antimicrobials. Calcium chloride may function by increasing solute concentration at the mushroom cap surface, making less water available to bacteria and increasing surface light reflectance (whiteness). In addition, it may improve vacuolar membrane integrity, making the mushroom tissue more resistant to bruising and senescence.

The high pH of the first-stage wash is designed to destroy bacteria on the mushroom cap surface, particularly the phytopathogenic fluorescent pseudomonads, which cause blotches and lesions. Erythorbic acid and sodium erythorbate, in addition to returning mushroom pH to physiological range, act as antioxidants, inhibiting enzymatic browning.

In addition to effectively improving the quality and shelf life of fresh mushrooms, high-pH/erythorbate treatment is useful as a pretreatment to improve the color of canned and frozen mushrooms.

This section presenting "Conclusions" of the inventors in the '507 patent corresponds precisely to the subject matter originally claimed and granted: use of a high pH solution to kill bacteria *followed by immediate use of an erythorbate buffer* to both neutralize the initial basic solution *and inhibit enzymatic browning.*

It should be noted, by the way, that despite the language used in the reissue declaration about being unduly limited, the patentee was not limited to the actual preferred embodiments in the originally granted patent. Erythorbate buffers are *required*, not preferred; preferred embodiments are mixtures of erythorbate buffers with other substances and/or use of erythorbate buffers at preferred concentrations.

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In addition to the "Conclusion" section recited above (in its entirety) that requires the use of an erythorbate buffer, the specification states at other locations that the comparative examples (including the one use of water after a high pH step) are *un*satisfactory. This is clearly shown in the following selections from the '507 patent, taken from the Summary of the Invention and Experimental sections:

> SUMMARY OF THE INVENTION
>
> The present invention provides a sulfite *alternative employing high pH (preferably 10.5-11.0) to control bacterial growth on mushrooms, and browning inhibitors to minimize enzymatic browning of mushroom tissue.*
>
> High pH (9.0 or above) has been shown to be effective for controlling the growth of bacteria in egg washwater (Catalano and Knabel, 1994). The present invention adapts high-pH solutions as an antimicrobial wash treatment for fresh mushrooms, to prevent bacterial decay of mushroom tissue and resultant tissue browning. With their high susceptibility to tissue damage, mushrooms represent a unique application of high-pH preservative treatments. Solution exposure time must be carefully controlled, to optimize bacterial destruction while avoiding counterproductive overexposure of mushrooms to extremes of pH, resulting in chemical damage to tissue. *Thus, the present invention comprises a multiple (two- or three-) stage wash procedure, with an initial high-pH antimicrobial step, followed by one or more pH neutralization/browning inhibitor washes, with an erythorbic acid/sodium erythorbate buffer with EDTA added, for example.*
>
> *The present invention provides a high-pH treatment for the control of bacterial spoilage of mushrooms. A first-stage, high-pH wash destroys bacteria, but might also directly damage mushroom tissue. This is avoided, however, if mushroom exposure time to the high-pH solution is brief and is followed immediately by a second-stage neutralization buffer, consisting primarily of the enzymatic browning inhibitors erythorbic acid and sodium erythorbate.*[9]
>
> Tribasic Sodium Phosphate Trials
>
> *In preliminary experiments*, solutions of tribasic sodium phosphate (trisodium phosphate, TSP), were used to generate a washwater pH of 11.0 or higher, as a one-stage wash or *in combination with water or the enzymatic browning inhibitors erythorbic acid or sodium erythorbate, in a second-stage wash solution.*

[9] '507 patent; col. 2, line 49, through col. 3, line 12.

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Use of 10% TSP by itself, in a wash lasting 120 seconds, was destructive to mushroom pileal tissue, yielding a Day 0 whiteness (L) value of 60.42, vs. 93.36 for a reverse-osmosis water wash and 95.10 for a 1000 ppm sodium metabisulfite wash (Appendix Table 1). TSP-washed mushrooms were dark brown in color and slimy in texture, compared to the bright white, dry, firm sulfite control mushrooms. Reduction of mushroom exposure time to TSP from 120 seconds to 60 seconds, followed by a reverse-osmosis-water wash of 60 seconds dramatically improved color, giving a day-0, L-value of 80.22.

Replacing water with a 2.25% sodium erythorbate solution in the second-stage wash yielded a further improvement in color, to an initial (Day 0) L-value of 89.23. When 2.25% sodium erythorbate was replaced with an equal concentration of erythorbic acid, initial whiteness was higher still, with a day-0, L-value of 90.71. Increasing erythorbic acid concentration from 2.25% to 4.50% gave very little improvement in color through day 3, but on day 6, the increased erythorbic acid treatment was noticeably better, with an L-value of 89.50, versus 84.12 for the 2.25% erythorbic acid treatment. Reduction of TSP concentration from 10% to 5% in the treatments with water as the second-stage wash improved color on days 0, 3, and 6.

None of the experimental treatments matched the whiteness of the sulfite and water controls through Day 3, but the two-stage treatment with 4.50% erythorbic acid as the second-stage wash was significantly better than the water-washed control and not significantly different from the sulfite-washed control on Day 6.

Development of a Two-Stage, High-pH/Neutralization Wash Treatment

Results of the trisodium phosphate wash trials indicated that the quality of mushrooms washed in basic-pH antibacterial solutions could be improved by subsequent transfer to a neutralization solution of erythorbic acid and sodium erythorbate. Erythorbate solutions acted as both an antioxidant, slowing the enzymatic browning reaction, and an acidulant, returning final mushroom pH to physiological range (approximately 6.5), thus minimizing tissue damage due to exposure to high pH.[10]

Language describing the two desired functions of the erythorbate buffer in the wash (immediately lowering pH while concurrently and immediately providing antibrowning effects) also appear at numerous other locations in the specification. The examiner is invited to review the specification as a whole, as well as the selections recited above, after which it will be apparent that the only workable process described in the

[10] '507 patent; col. 8, line 57, through col. 9, line 27.

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'507 patent is one that uses an erythorbate buffer wash immediately after the high pH antibacterial step.

It should also be noted that "immediate" use of an erythorbate buffer (i.e., without an intervening wash step and without intervening time) is all that is disclosed in the '507 specification. No intervening step is every shown or described. *Immediate* use of erythorbate buffer is critical, as stated by the patentee:

> A first-stage, high-pH wash destroys bacteria, but might also directly damage mushroom tissue. *This is avoided, however, if* mushroom exposure time to the high-pH solution is brief and is followed *immediately* by a second-stage neutralization buffer, consisting primarily of the enzymatic browning inhibitors erythorbic acid and sodium erythorbate.[11]

Since the process is designed to avoid damaging mushrooms and since the high pH wash can damage them, "immediate" application of an erythorbate buffer that avoids both high pH damage and browning produces satisfactory results. There is no indication anywhere in the specification that the desired elimination of both high pH damage and mushroom browning (two different processes, as explained in the specification) can be avoided without "immediate" use (i.e., without intervention of either time or another step) of an erythorbate buffer.

That the '507 patent does not describe a process of lowering pH with acidulants other than an erythorbate buffer, followed by a third step using a combination of any browning inhibitor (including an erythorbate buffer) and a chelating agent (such as EDTA), is a decision already reached by the USPTO. The EPL '476 patent was recently granted with the following broadest claim:

> 1. A method for preserving mushrooms comprising the steps of:
>
> contacting the mushrooms with an aqueous anti-microbial solution having a pH of from about 10.5 to about 11.5;
>
> rinsing the mushrooms at least once with at least one aqueous pH neutralizing buffer solution comprising organic acid and at least one salt

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[11] '507 patent; col. 3, lines 5-12.

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of an organic acid and substantially free from erythorbic acid and sodium erythorbate; and

contacting the mushrooms at least once with at least one solution comprising a browning inhibitor and a chelating agent.[12]

If such a process had been described in the specification of the '507 Penn State patent, the '476 EPL patent should not have been granted. However, the '476 patent was granted, despite the '507 patent having been considered specifically as prior art against the '476 patent (see the face of the '476 patent). Since the '507 patent clearly shows immediate use of an erythorbate buffer and the chelating agent EDTA (see example in column 10 of the '507 specification), there has already been a USPTO decision that the use claimed by EPL in the '476 patent (i.e., use of other acidulants to neutralize the first high pH step, followed by a third wash with an erythorbate solution, or another material that acts as an antioxidant, along with a chelating agent) is not disclosed in the '507 patent.

The patentee in the '507 reissue may wish that the specification discloses something beyond its original content, but a mere wish to encompass additional subject matter does not entitle a patentee to broadened reissue claims that have been expanded beyond the scope of the original disclosure so that they have an unwarranted monopolistic economic effect.

CONCLUSION

That which is not disclosed and enabled cannot be claimed. The '507 patentee is attempting to enlarge its claims to cover subject matter not originally disclosed or enabled in order to obtain an economic advantage to which it is not entitled, based on the specification in question. Such a request should be denied.

If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned at his direct telephone extension: (650) 843-5070.

[12] '476 patent; col. 18, lines 54-64.

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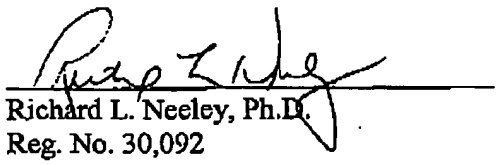
By: 
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(12) United States Patent
Martin et al.

(10) Patent No.: US 6,500,476 B1
(45) Date of Patent: Dec. 31, 2002

(54) PRESERVATION COMPOSITIONS AND PROCESS FOR MUSHROOMS

(75) Inventors: Stefan T. Martin, Flourtown, PA (US); Howard S. Kravitz, Wayne, PA (US); William R. Romig, Moorestown, NJ (US)

(73) Assignee: EPL Technologies, Inc., Philadelphia, PA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: 09/896,869

(22) Filed: Jun. 29, 2001

Related U.S. Application Data

(63) Continuation-in-part of application No. 09/819,857, filed on Mar. 28, 2001.

(51) Int. Cl.[7] A23B 7/153

(52) U.S. Cl. 426/262; 426/271; 426/310; 426/335; 426/615

(58) Field of Search 426/615, 262, 426/541, 335, 532, 648, 102, 49, 321, 326, 329, 302, 271

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(57) ABSTRACT

Preservative compositions using GRAS (generally recognized as safe) ingredients are incorporated into a commercially viable three-stage preservation process for mushrooms. The process includes contacting the mushrooms with a high pH solution for microbial reduction; a neutralizing step to return the pH of the mushrooms to their about physiological pH; and an anti-browning step that incorporates suitable antioxidants and ancillary compounds to maintain the color of the finished product. Specifically disclosed is a method for preserving fresh mushrooms comprising the steps of exposing the mushrooms to a aqueous antimicrobial solution with a pH of 10.5–11.5; treating the mushrooms with a pH neutralizing buffer consisting of an organic acid and a salt of an organic acid and then subjecting the mushrooms to a combination of an anti-browning agent, a source of calcium and a chelating agent.

32 Claims, 2 Drawing Sheets

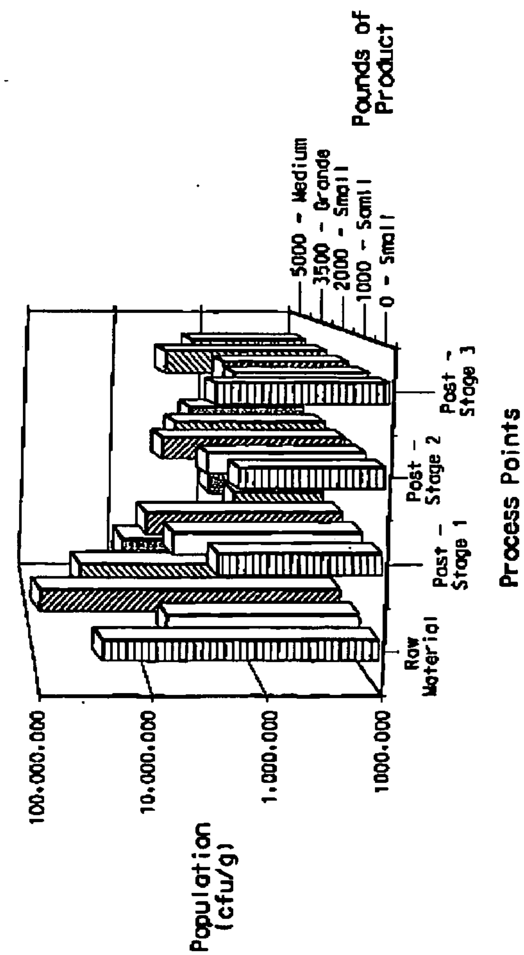

FIG. 1

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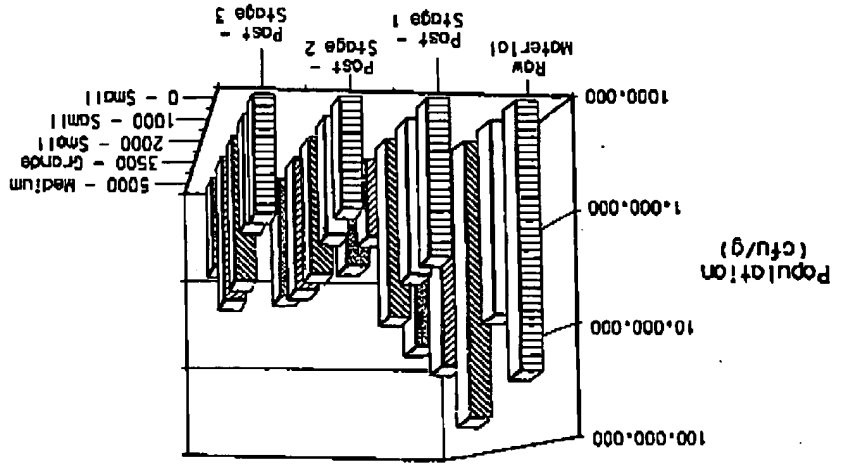
FIG. 2



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Specifically, the instant invention provides a method for preserving mushrooms comprising the steps of contacting the mushrooms with an antimicrobial solution having a pH from about 10.5 to about 11.5; rinsing the mushrooms at least once with at least one aqueous pH neutralizing solution comprising organic acid and at least one salt of an organic acid substantially free from erythorbic acid and sodium erythorbate; and contacting the mushrooms at least once with at least one solution comprising a browning inhibitor and a chelating agent.

The invention further provides a three stage process that includes calcium and EDTA to minimize enzymatic browning in the third stage.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1 and 2 are graphical representations of test results realized using the three-stage process of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the discovery that a three stage preservation method for mushrooms provides improved efficiency and efficacy in cleaning and preserving mushrooms on a commercial scale.

The present invention uses a high pH solution as an anti-microbial treatment for fresh whole or sliced mushrooms. This significantly reduces the microbial load and reduces bacterial decay of the mushroom tissue and subsequent browning. However, the mushroom cap tissue is susceptible to damage by exposure to the high pH solution. Therefore, the present process includes a rapid neutralization following the high pH wash. The rapid neutralization is important to the quality of the finished product and the exposure time should be carefully controlled.

The present invention also includes an antibrowning step to deal with enzymatic browning. The antibrowning step incorporates a solution comprising antioxidant or browning inhibitor, and preferably calcium to maintain cellular tissue and to enhance browning inhibition. EDTA is also preferably used to further increase browning inhibition. By separating the neutralization step and the anti-browning step, the process is made most effective due to the reduced depletion of the relatively expensive anti-browning solution. Thus, the present invention consists of three distinctly functional stages: a microbial stage, a neutralization stage and an anti-browning stage.

The first step of the instant process involves contacting the mushrooms with an anti-microbial buffer solution having a pH of from about 10.5 to about 11.5. A wide variety of compounds can be used in this solution to attain the desired pH, including, for example, sodium bicarbonate, sodium carbonate and sodium hydroxide. The buffering components can be used alone or in combination. Of these buffering compounds, the combination of sodium bicarbonate and sodium carbonate is preferred. About from 0.3 to 0.5% by weight sodium bicarbonate and about from 0.05 to 0.10% by weight of sodium bicarbonate has been found to be particularly satisfactory. In general, the initial contacting with the anti-microbial buffer solution is carried out for about from 20 to 40 seconds at ambient temperatures of about 25° C. Somewhat elevated temperatures can provide greater anti-microbial action, which permits lower dwell times in this solution.

The mushrooms are thereafter contacted with at least once with at least one aqueous pH neutralizing buffer solution comprising organic acid and at least one salt of an organic acid and substantially free from erythorbic acid and sodium erythorbate. This can be accomplished by applying the buffer solution by any convenient means, including, for example, by dipping, spraying and cascading. In general, this neutralizing solution has a pH of about from 3.0 to 5.0. The acids and bases used for the preparation of the salts are preferably weak acids and bases. Representative of the organic acids and salts of organic acids which can be used in the instant process include citric acid and sodium citrate, respectively. For example, a 0.1 N solution of citric acid, having a pH of about 3.5, can be used effectively. The combination of citric acid and sodium citrate is preferred, but other acids, salts, and combinations will be readily apparent to those skilled in the art. For example, other organic acids which can be used include malic, acetic, phosphoric, and lactic acids.

The rinsing step is carried out to reduce the pH of the mushrooms to substantially their natural pH. In this context, the rinsing is carried out with the aqueous buffer solution at a pH of about from 3.0 to 5.0. The contacting time will vary, for example, with the pH of the mushrooms after the anti-microbial solution treatment and the volume of rinsing solution, but is typically about from 10 to 30 seconds.

The third step of the process of the instant invention is treating the mushrooms at least once with at least one solution comprising a browning inhibitor and a chelating agent. A wide variety of browning inhibitors known in the art can be used, which retard the effect of tyrosinase. These are typically reducing agents, of which sodium erythorbate, erythorbic acid, ascorbic acid and calcium ascorbate are representative, and which are preferred. A similarly broad range of known chelating agents can be used, which have a high affinity for copper. These can include, for example, polyphosphates such as sodium hexametaphosphate, and others currently approved for use on fruits and vegetables, and categorized by the Food and Drug Administration as Generally Recognized As Safe (GRAS). Calcium disodium EDTA has been found to be particularly satisfactory, and is accordingly preferred.

The pH of individual solutions can be monitored for the purpose of maintaining the optimum pH and the concentration of sodium erythorbate monitored to maximize the inhibition of enzymatic browning of the mushrooms. In a preferred embodiment of the instant process, the solution used in the third treating step further comprises calcium chloride. The pH of individual solutions is monitored for the purpose of maintaining the optimum pH and the concentration of sodium erythorbate monitored to maximize the inhibition of enzymatic browning of the mushrooms.

Advantages of the present process over other multiple stage processes include the use of higher pH solutions in the first stage, resulting in improved microbial kill, a rapid neutralization step that minimizes tissue damage, less carry over and a reduction in the rate of depletion of the expensive anti-browning chemicals, better dirt removal, less solution uptake (high solution uptake causes the appearance of light gray discoloration, commonly referred to as "window paning"), reduced tank charging costs, reduced ingredient depletion and less foam in the final tank.

The instant process preferably comprises incorporating, into at least one treating step, at least one antifoaming composition. A wide variety of known defoaming or anti-foaming compositions or surfactants can be used, of which silicon emulsions have been found to be particularly satisfactory, and are accordingly preferred. For maximum effectiveness, this component is preferably added to the first or second stages of the instant process. The specific quantity will depend on the surfactant selected and the volume of the treating baths, as can be readily determined by those skilled in the art.

The procedure can be and preferably is a continuous process whereby the mushrooms are introduced into a first tank and conveyed through each step with minimal mechanical damage. Excessive mechanical damage leads to excessive browning and depletion of active ingredients. Solutions of sodium bicarbonate and sodium carbonate are adjusted to the higher pH with sodium hydroxide to achieve the high pH state in the first stage and maintained at a temperature of at least about 25° C. In general, somewhat elevated temperatures improve the microbial kill. To avoid tissue damage, the exposure time is limited to about 20–40 seconds. After the antimicrobial step, the pH of the mushrooms is rapidly adjusted to approximately 6.5, which is more physiologically acceptable for the mushrooms. The rapid reduction in pH is accomplished during the second step in the process or the rinsing step. The rinsing occurs in a tank that contains a citrate buffer made from an organic acid and a salt of an organic acid and that is at ambient temperature. To minimize the uptake of solution, the mushrooms remain in stage two for only approximately 10–30 seconds. They are then transported by a conveyor that minimizes submersion depth, again to minimize uptake of solution, to the stage three (antibrowning) step. The antibrowning stage solution is maintained at ambient temperature and preferably comprises sodium erythorbate, calcium chloride, and EDTA as a treatment to minimize enzymatic browning. The mushrooms remain in this solution for approximately 20–40 seconds. The total immersion or solution exposure time during the three stage process is preferably limited to approximately 50–110 seconds.

When compared to other treatment protocols such a sodium metabisulfite or the Beelman-Duncan two stage process, mushrooms resulting from the process of the instant invention are superior in several ways. Although mushrooms treated with sulfites may initially have a better whiteness than mushrooms treated by the present invention, those resulting from the present invention exhibit a better appearance later in shelf life and exhibit a longer shelf life with acceptable color. There is a significant reduction in microbial load using this process whereas sulfites tend to exhibit little or no bactericidal effect. When compared to the Beelman-Duncan two stage process, mushrooms from the present three-stage process take up less solution, have a greater reduction in microbial load, and exhibit less enzymatic browning. The three distinct steps allow for the optimization of each functional component of the process and minimization of the depletion of active components in the anti-browning stage. This results in a significant reduction in cost of the process per pound of treated mushrooms.

The present invention is further illustrated in the following specific examples, using the materials, equipment and test procedures described.

Raw Material

Hybrid off-white mushrooms (*Agaricus Bisporus*) were used for testing. Due to the inherent variability in commercially produced mushrooms all subsequent studies were conducted with sterile mushrooms but grown and harvested under commercial production conditions in southeastern Pennsylvania. No grading was done beyond normal commercial protocols. Traditional horse-manure compost and conventional production practices were used. Mushrooms were generally harvested early in the day of processing. A composite of the harvest was used with the only differentiation being the harvest or flush as it is commonly referred to in the industry. As the teaching of this invention is a commercially viable process the use of average quality mushrooms was considered very important. First and second flush mushrooms, which account for the majority of mushrooms sold commercially were used for the pilot scale tests and within a given test either the first or second flush mushrooms were used. Harvested mushrooms were stored at 2–4° C. prior to processing. Experiments were conducted with whole and sliced mushrooms.

Treatment Solutions

Treatment solutions were generally made using available city or well water. However, the water was analyzed to determine the pH and any unusual concentrations of specific elements.

Initial studies and early pilot scale tests compared the Beelman et al. two-stage process and the present three-stage process. All subsequent wash treatments where commercially grown mushrooms were used consisted of three distinct stages: the first stage is a high pH antimicrobial wash (typically 10.5–11.5 sodium bicarbonate, sodium carbonate buffer), the second stage is a neutralization step to return the mushrooms to the physiologically normal pH as quickly as possible, the third stage is a preservative step (typically a mixture of sodium erythorbate, calcium chloride, and EDTA). It is extremely important to neutralize the high pH solution quickly to avoid tissue damage and subsequent accelerated enzymatic browning and microbial growth.

Stage one (reported in Table 1) uses a combination of 0.04M sodium carbonate and 0.01M sodium bicarbonate adjusted to pH 11.2 with 10M sodium hydroxide. A surfactant is added to avoid foaming in the treatment tank and subsequent carry over to tank two. The temperature of the solution is maintained at 25° C.

Stage two neutralization solution is a 0.05M citrate buffer of citric acid and sodium citrate to achieve a pH range of 2.5–4.0 with a pH of 3.6 being the target. The temperature of this solution is ambient.

Stage three anti-browning step consisted of sodium erythorbate (2.0–3.0% by weight), calcium disodium EDTA (0.1% by weight) and anhydrous calcium chloride (0.1–0.2% by weight). The temperature of stage 3 is ambient.

The pH of individual solutions is monitored for the purpose of maintaining the optimum pH and the concentration of sodium erythorbate monitored to maximize the inhibition of enzymatic browning of the mushrooms.

Wash Process

Three commercial size tanks (FIG. 1) were filled with solution prepared with plant water. The temperature of tank 1 is maintained at 25° C. while the temperature of tank 2 and tank 3 equilibrated to ambient conditions. Chemical solutions and conditions used in the individual tanks are summarized in table 1. The single stage process is the current practice of using sulfite solutions to bleach and preserve the mushrooms. The two-stage process is the process developed by Beelman and Duncan.

Mushrooms are dumped gently into Tank 1 and conveyed gently through the tank at the designated rate via paddles. The process line is a continuous process with tank-to-tank transitions as gentle as possible and with minimal submersion to minimize solution uptake and damage to the mushrooms. Excess moisture was drained during the transition from Tank 1 to Tank 2, which helped to minimize carryover into Tank 1.

Quality Measurements

Effectiveness of the individual treatments at minimizing whiteness and inhibiting post-processing browning was determined by measuring mushroom cap color on designated days of storage. Color was measured at three locations on the surface of each mushroom cap using either human observation or a tristimulus colorimeter. The colorimeter was calibrated using a standard plate supplied with the instrument and L* a*b color coordinates were used for all measurements. A target color of L=97.00, a=2.00, and b=0.00 was used as a reference standard for internal calculation of overall color deviation (Delta E) from that of the "ideal white mushroom" (Solomon, 1991).

In some studies, cap (visual), stem color and texture were rated on a 1 to 5 scale with 1 being the best. Notations were made for aroma and extent of visible microbial degradation.

TABLE 1

	Single-Stage Process	Two-Stage Process	Three Stage Process
Tank 1	Water Sodium Metabisulfite Acidified Temperature	Water Sodium bicarbonate pH 9.5–11.0 30–60 sec 30–35 C.	Water Sodium bicarbonate Sodium carbonate Sodium hydroxide sufficient pH 10.5–11.0 30 sec 15 C.
Tank 2	None	Water Erythorbic acid Sodium erythorbate Calcium disodium EDTA Anhydrous Calcium Chloride pH 4.8 60–120 sec exposure	Water Citric acid Sodium citrate pH 3.8–4.4 15 sec Surfactant
Tank 3	None	None	Water Sodium erythorbate Calcium disodium EDTA Anhydrous calcium chloride Surfactant pH 7.5 15 sec dwell time

EXAMPLE 1

Reducing uptake of solution and minimizing depletion of the preservative solution in tank 3.

Treatment solutions were prepared with deionized water. Stage 1 was maintained at 25° C. for multiple stage wash procedures while the sulfite control was at ambient temperature. The objective was to identify a process that would meet the economic targets of the processor and yield results (quality and shelf life) as good or better than the two-stage process reported by Beelman and Duncan. The mushrooms were held at 10° C. Three-stage processes (treatments 5, 7, 9) yielded results as good as or better than the best 2-stage process with regard to overall quality and shelf life. Treatment 7 was the best with regard to appearance due in part to the reduced exposure time and less uptake of solution.

TABLE II

Treatment	Description	Color of Stored Mushroom (L* values) Day 0	Day 3	Day 6
1	Sodium bicarbonate buffer at pH 10.5 (30 sec); 1% erythorbic acid sodium erythorbate + 1000 ppm EDTA + 1000 ppm calcium chloride (60 sec)	94.14	84.24	84.83
2	Sodium bicarbonate buffer at pH	94.34	91.43	90.00

TABLE II-continued

Treatment	Description	Color of Stored Mushroom (L* values) Day 0	Day 3	Day 6
	10.5 (30 sec), 0.05M citrate buffer @ pH 3.4 (15 sec); 1.0% sodium erythorbate + 1000 ppm EDTA + 1000 ppm calcium chloride (15 sec)			
3	Sodium bicarbonate buffer at pH 10.5 (30 sec); 0.05M citrate buffer @ pH 4.4 (15 sec); 1.0% sodium erythorbate + 1000 ppm EDTA + 1000 ppm calcium chloride (15 sec)	91.97	89.84	88.05
4	Sodium bicarbonate buffer at pH 10.5 (30 sec); 0.05 citrate buffer at pH 3.4 (15 sec); 1.5% sodium erythorbate + 1000 ppm EDTA + 1000 ppm calcium chloride (15 sec)	94.56	94.22	91.85
5	Sodium bicarbonate buffer at pH 10.5 (30 sec), 0.05M citrate buffer at pH 4.6 (15 sec); 1.5% sodium erythorbate + 1000 ppm EDTA + 1000 ppm calcium chloride (15 sec)	94.72	93.38	93.45
6	Sodium bicarbonate buffer at pH 10.5 (30 sec); 0.05M citrate buffer at pH 3.4 (15 sec); 1.0% sodium erythorbate + 1000 ppm EDTA + 1000 ppm calcium chloride (15 sec)	94.81	91.45	89.70
7	Sodium bicarbonate buffer at pH 10.5 (30 sec); 0.05M citrate buffer at pH 4.2 (15 sec); 2.0% sodium erythorbate + 1000 ppm EDTA + 1000 ppm calcium chloride (15 sec)	94.51	94.76	93.82
8	Same as treatment 5 but with EDTA in the second (citrate) wash instead of in the third (erythorbate) wash.	92.31	93.14	88.83
9	Same as treatment 5, but with the citrate and sodium erythorbate washes at 30 sec each instead of 15 sec for a total exposure time of 90 sec.	93.43	93.38	91.91
10	Sodium bicarbonate buffer at pH 10.5 (30 sec); 0.05M citrate buffer at pH 3.4 (15 sec); 3.0% sodium erythorbate + 1000 ppm EDTA + 2000 ppm calcium chloride (15 sec)	95.81	93.40	91.90

EXAMPLE 1, CONTINUED

Effect of washing on quality parameters of mushrooms Day 6. (1=best, 5=worst)

Treatment	Description	Cap Color	Stem Color	Areas	Maturity	Microbial
1 Control	Sodium bicarbonate buffer at pH 10.5 (30 sec); 3% erythorbic acid/sodium erythorbate + 2000 ppm EDTA + 2000 ppm calcium chloride (60 sec)	1	1		3	

-continued

Treatment	Description	Cap Color	Stem Color	Areas	Maturity	Microbial
2	Sodium bicarbonate buffer at pH 10.5 (30 sec); 0.05 M citrate buffer @ pH 3.4 (15 sec); 1.0% sodium erythorbate + 1000 ppm EDTA + 1000 ppm calcium chloride (15 sec)	3	3 dark	Many	2	Dramatic black
3	Sodium bicarbonate buffer at pH 10.5 (30 sec); 0.05 M citrate buffer @ pH 4.6 (15 sec); 1.0% sodium erythorbate + 1000 ppm EDTA + 1000 ppm calcium chloride (15 sec)	4 dark edges	3 brown	SL Tulip	1	Some black
4	Sodium bicarbonate buffer at pH 10.5 (30 sec); 0.05 citrate buffer at pH 3.4 (15 sec); 1.5% sodium erythorbate + 1000 ppm EDTA + 1000 ppm calcium chloride (15 sec)	1	1	SL Anyamels	3	black
5	Sodium bicarbonate buffer at pH 10.5 (30 sec); 0.05 M citrate buffer at pH 4.6 (15 sec); 1.5% sodium erythorbate + 1000 ppm EDTA + 1000 ppm calcium chloride (15 sec)	1	1		3	
6	Sodium bicarbonate buffer at pH 10.5 (30 sec); 0.05 M citrate buffer at pH 3.4 (15 sec); 1.0% sodium erythorbate + 1000 ppm EDTA + 1000 ppm calcium chloride (15 sec)	3 sl. darkening	3 browning	Flaky	4	Some black
7	Sodium bicarbonate buffer at pH 10.5 (30 sec); 0.05 M citrate buffer at pH 4.6 (15 sec); 2.0% sodium erythorbate + 1000 ppm EDTA + 1000 ppm calcium chloride (15 sec)	1	1		2	
8	Same as treatment 5 but with EDTA in the second (citrate) wash instead of in the third (erythorbate) wash.	2.5 sl. White	1.5	Flaky	3	black
9	Same as treatment 5, but with the citrate and sodium erythorbate washes at 30 sec each instead of 15 sec for a total exposure time of 90 sec.	2	2		3	black
10	Sodium bicarbonate buffer at pH 10.5 (30 sec); 0.05 M citrate buffer at pH 3.4 (15 sec); 3.0% sodium erythorbate + 2000 ppm EDTA + 1000 ppm calcium chloride (15 sec)	1	2.5 darkening	Flaky	4	Some black

EXAMPLE 2

The 3-stage process (treatment 7) yielded mushrooms of equal or better quality than the standard 2-stage process as illustrated by L*-values (whiteness readings). The reduced exposure time also limited solution uptake, which is detrimental to quality and shelf life.

Treatment	Description	Mushroom Color (L* values) Day 0	Day 3	Day 6
1	Sodium sulfite—Control	91.48	90.89	87.28
2 Two-Stage Control	Sodium bicarbonate buffer at pH 10.5 (30 sec); 3.0% erythorbic acid/sodium erythorbate + 1000 ppm EDTA + 1000 ppm calcium chloride (60 sec)	93.88	91.13	91.46

-continued

Treatment	Description	Mushroom Color (L* values) Day 0	Day 3	Day 6
3	Sodium bicarbonate buffer at pH 10.5 (30 sec); dip mushrooms in 0.05M citrate buffer at pH 3.5 (15 sec); 3% sodium erythorbate + 1000 ppm EDTA + 1000 ppm calcium chloride (15 sec)	92.70	90.94	91.25
4	Same as treatment 3, but with a 5 sec water rinse at end	90.70	90.31	89.18
5	Same as treatment 3, but with 1.5% sodium erythorbate instead of 3.0%	93.63	90.89	89.73
6	Same as treatment 3, but with a 5 sec water rinse at the end	90.49	88.55	90.00
7	Same as treatment 3, but with a	93.67	93.02	91.49

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-continued

Treatment	Description	Day 0	Day 3	Day 6
	rinsing time of 30 sec in sodium erythorbate solution, instead of 15 sec			
8	Same as treatment 7, but with a 5 sec water rinse at the end	91.14	92.29	91.79

(The last three columns are headed "Browning Color (L* value)".)

EXAMPLE 3

Three-stage treatment illustrating equal or superior performance with reduced exposure times and reduced concentration of erythorbate resulting in an acceptable processing cost. Three-stage treatments 2, 3, 5 and 6 all outperformed the two-stage process over the duration of the shelf life period as illustrated by the higher L* value readings. The advantage of the 3-stage process is at day 6 when the retail consumer typically sees the product.

Treatment and Description		Day 0	Day 3	Day 6
1	Sodium bicarbonate buffer at pH 10.5 (30 sec); 3% erythorbic acid/sodium erythorbate + 1000 ppm EDTA + 1000 ppm calcium chloride (30 sec) (standard 2-stage process)	94.50	93.42	93.13
2	Sodium bicarbonate buffer at pH 10.5 (30 sec), 0.05 citrate buffer at pH 4.4 (15 sec); 1.8% erythorbate + 1000 ppm EDTA + 1000 ppm calcium chloride (15 sec)	93.67	92.31	91.73
3	Sodium bicarbonate buffer at pH 10.5 (30 sec), 0.05M citrate buffer at pH 4.1 (15 sec), 3.0% erythorbate + 1000 ppm EDTA + 1000 ppm calcium chloride (15 sec)	94.33	93.30	92.93
4	Sodium bicarbonate buffer at pH 10.5 (30 sec); 0.05M citrate buffer at pH 3.4 (15 sec); 1.5% erythorbate + 1000 ppm EDTA + 1000 ppm calcium chloride (15 sec)	93.43	92.94	91.93
5	Sodium bicarbonate buffer at pH 10.5 (30 sec); 0.07M citrate buffer at pH 4.4 (15 sec); 1.5% erythorbate + 1000 ppm EDTA + 1000 ppm calcium chloride (15 sec)	93.78	93.10	91.36
6	Sodium bicarbonate buffer at pH 10.5 (30 sec); 0.05M citrate buffer at pH 3.4 (15 sec); 2.0% erythorbate + 1000 ppm EDTA + 1000 ppm calcium chloride (15 sec)	94.84	93.20	91.49
7	Sodium bicarbonate buffer at pH 10.5 (30 sec); 0.05M citrate buffer at pH 4.6 (15 sec); 2.0% erythorbate + 1000 ppm EDTA + 1000 ppm calcium chloride (15 sec)	93.13	92.30	90.84
8	Same as 4 but with EDTA in second (citrate) tank instead of the third (erythorbate) tank.	93.82	92.41	91.14

EXAMPLE 4

Comparison of the Brekman-Duncan process and the present three-stage process using mushrooms grown under conventional production protocols and washed using a commercial processing line.

First-flush mushrooms were treated with several variations of the three-stage process as well as the standard two-stage process of Brekman and Duncan. The control was

commercially produced sulfite-washed mushrooms. Tank 1 was 0.424% sodium carbonate, 0.084% sodium bicarbonate buffer (0.05M), pH=10.5, temperature 25° C.; tank 2 was 0.49% citric acid, 0.72% sodium citrate buffer, pH 4.5, temperature 14° C.; tank 3 was 3.0% sodium erythorbate (monohydrate), 1000 ppm calcium disodium EDTA, 1000 ppm calcium chloride (dihydrate), pH 7.2–7.4. After washing, the mushrooms were placed in cold storage prior to vacuum cooling. Mushrooms were vacuum cooled for 18 min to an internal temperature of 2–3° C. Mushrooms were packaged in commercial 10-LB ventilated corrugated containers. Mushrooms treated with the various three-stage treatments retained shelf life quality as well as those treated with the commercial sulfite solution. There were no major differences across the different three-stage treatments and the mushrooms exhibited an additional 2–3 days of salable quality. The three-stage treatments were superior to the standard two-stage process. Overall ranking scale (1=best) was used to compare the processes.

The fact that the 3-stage process worked as well with regard to appearance as the commercial sulfite wash (banned by FDA) and provided additional shelf life indicated that this process performs adequately for commercial use. Results are especially significant in that the starting raw material was first-flush mushrooms, which are generally of lower quality than second flush mushrooms.

Evaluation of mushrooms processed and stored under commercial conditions for 3 days at 2–4° C. The plant manager performed evaluations. An overall ranking scale (1=best) was used to compare the processes.

Treatment	Cap Color	Stem End Color	Overall Salability	Comments	Overall Ranking
3-stage #1 30s/15s/15s	Better than sulfite control	Equal to or better than sulfite control	Salable	Good throughout	1
3-stage #2 30s/30s/15s	Some blotch staining, but no different than sulfite control	Equal to sulfite control	Salable	Some dimpling and blotch	4
3-stage #3 30s/15s/30s	A little better than sulfite control	Equal to sulfite control	Salable		2
3-stage #4 30s/30s/30s	A little better than sulfite control	Not as good as sulfite control	Salable		3
3-stage #5 30s/15s/30s with rinse	Caps dirty, equal to control	Equal to sulfite control	Salable		4
3-stage #6	More blotch than sulfite control, not as good	Equal to sulfite control	Marginal	A lot of blotch	5
Sulfite Control	Acceptable	Acceptable	Salable		6

EXAMPLE 5

First and second flush, calcium chloride-irrigated mushrooms were washed in the standard 3-stage process, a modified 2-stage process in which the second and third steps were combined and a bucket test to evaluate the significance of the mechanical damage. Mushrooms washed in the bucket test of the 3-stage process were rated "excellent" and almost as good as sulfite-washed mushrooms. Those washed with the standard 3-stage process were rated "good" by day 2 and were still acceptable to marginal by day 6, but were

not as bright as those from the bucket test. However, mushrooms treated via the 3-stage process were considerably brighter than mushrooms washed with the 2-stage process, thus confirming the need to separate the functionalities of the ingredients in separate tanks.

Standard 3-stage process:

Stage 1—40 sec (target is 30 sec) in 0.424% sodium carbonate, 0.084% sodium bicarbonate at approx. 25° C., pH 10.5

Stage 2—45 sec (target time 15 sec) in 0.49% citric acid, 0.72% sodium citrate at approx. 16° C., pH 4.6

Stage 3—16 sec (target time 15 sec) in 3.0% sodium erythorbate, 0.1% calcium disodium EDTA, 0.1% calcium chloride at approx. 16° C., pH 7.3

Post-treatment rinse—1 oz/LB

Modified 2-stage process

To further confirm the importance of separating the functional steps of the wash process a modified 2-stage process was tested where steps 2 and 3 of the 3-stage process were combined.

Stage 1—45 sec (target time 30–45 sec) in 0.424% sodium carbonate, 0.084% sodium bicarbonate at approx 25° C., pH 10.5.

Stage 2—16 sec (target time 15 sec) in 3.0% sodium erythorbate, 0.1% calcium chloride, 0.1% calcium disodium EDTA, and adjusted to pH 4.8 with citric acid

Post-treatment rinse—1 oz/LB

2-Stage Process (stages 2 and 3 of three-stage process combined)	Product rated acceptable to marginal. Not as bright as mushrooms from the 3-stage process, significantly more bruising
3-Stage Process	Product rated acceptable to marginal. Caps mostly white with only a few ending then with had good color
3-stage bucket test	Mushrooms from 2nd flush about as good as those from the 3-stage process (above), but those from the 1st flush were unacceptable due to lots of deep brown cracking, probably due to over dipping

EXAMPLE 6

A three-stage process using sprays for stage 2 and stage 3 rather than submersion.

Mushrooms of four different quality levels were washed using the three-stage process but with the substitution of sprays for tank 2 and tank 3. Finished product was stored at 35° F. and compared with unwashed controls and sulfite-washed mushrooms.

Mushroom Quality (starting raw material)

Group 1: Second break, medium-size mushrooms (best quality of the four groups), picked and washed the same day.

Group 2: Second break medium-size mushrooms picked same day, in 10-LB tills, vacuum cooled (second best quality)

Group 3: California first break medium-size mushrooms picked the previous day, in lugs, vacuum-cooled (worst quality of the four categories)

Group 4: First break medium-size mushrooms picked same-day, in lugs, not vacuum-cooled.

Parameters for trial

Stage 1—15–30 sec at 25° C. in 0.424% sodium carbonate, 0.084% sodium bicarbonate (pH 10.4)

Stage 2—20 sec at 13° C. in 0.72% sodium citrate, 0.49% citric acid

Stage 3—10 sec at 13° C. in 3.5% sodium erythorbate, 0.2% calcium chloride and 0.1% calcium disodium EDTA

The mushrooms were evaluated by technical personnel from a commercial mushroom processor.

Mushrooms were kept at room temperature for approximately 30 min after washing, then placed in the 35° F. cooler. Lugs were stacked and uncovered. Control (unwashed and sulfited mushrooms) was placed next to the treated material.

Results at Day 3

Group 1—Washed product looked good, caps were white on top, slightly gray around sides. Slightly better than sulfite-washed same day; sulfite-washed were slightly yellow. Stem ends ok, slight to moderate brown.

Group 2—3-stage wash product looked good, some bruising, otherwise as good as group 1.

Group 3—3-stage process looked marginal as they did on day 0. Acceptable for slicing.

Group 4—3-stage process looked acceptable to marginal, lot of bruising, overall not as bright as groups 1 and 2.

Although there was considerable mechanical damage the 3-stage processed mushrooms were acceptable and considered better than the sulfited control.

Day 5

The 3-stage processed mushrooms still looked good with little difference from day 3. The treated product was slightly better than the unwashed controls and significantly better than the sulfite-washed product, which continued to yellow.

Day 7

Treated product still looked good. The 3-stage washed product clearly looked better than the unwashed controls and much better than the sulfite-washed mushrooms. There was some blotch on the unwashed controls but none on the washed mushrooms.

EXAMPLE 7

Three-stage Treatment Trial Substituting Spray Application for Submersion in stages 2 and 3

Treatments were the same as in Example 6.

This was a similar trial to that described in Example 5 but with lesser quality starting raw material. Mushrooms were dumped onto a metal grill (slopes to tank 1) to remove debris. The mushrooms remained in the barrel washer for 15–30 seconds. Stage 2 consisted of spray nozzles mounted over a conveyor and supplied by a 50-gal tank. Total time for stage 2 was 20 sec. Stage 3 also consisted of spray nozzles over a second conveyor and fed by a 50-gal tank. The mushrooms were allowed to drain and were sorted for defects and size.

In general the results were not as good due to the inferior quality of the starting raw material. None of the mushrooms in this trial were of superior quality and some were clearly marginal quality at the time of washing. Three of the six tested groups were harvested the day before.

Day 7 Results

pallet 1 (large, second break, harvested day before washing, decent quality prior to washing)

Sliced—excellent quality (rated 8-9 on scale of 1 to 10, 10 being best)

Whole—good quality—biggest problem was brown spots due to mechanical damage (rated 7)

Pallet 2 (medium, second break, harvested day before washing, fair quality prior to washing)

Sliced—excellent quality (rated 9)

Whole—good quality—biggest problem was brown spots due to mechanical damage (rated 7)

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Unwashed Controls—were brown overall (rated 3)

Pallet 3 (medium, first break, harvested day before washing, low quality prior to washing)

Sliced—good quality (rated 7 on a scale of 1 to 10)

Whole—decent quality (rated 6 on a scale of 1 to 10)

Unwashed controls—somewhat brown overall (rated 4)

Pallet 4 (medium size, first break, picked day of wash, decent quality prior to washing)

Sliced—very good quality (rated 8)

Whole—good quality—browning where there was mechanical damage (rated 7)

Unwashed controls—a little brown overall (rated 5)

Pallet 5 (buttons, picked day of wash, low quality prior to washing)

Whole—marginal quality (rated 5)

Pallet 6 (medium, first break, picked day of washing, good quality prior to washing)

Sliced—marginal—unacceptable quality, a lot of graying (rated 4)

Whole—decent quality (rated 6)

EXAMPLE 8

Effect of Three-stage Process on Depletion of Active Ingredients

The largest cost factor in the two-stage process is erythorbate, added as sodium erythorbate and erythorbic acid. The largest cost factor in the three-stage process is sodium erythorbate. The concentration of erythorbate must be maintained between approximately 2 and 3% in order to effectively preserve the appearance of the washed mushrooms. Therefore, the depletion and maintenance of erythorbate is a critical component in both efficacy and the economics of both wash processes.

Mushrooms were washed in separate commercial trials of the two-stage and three-stage processes. The changes in concentration of erythorbate were monitored in tank 2 of the two-stage process and tank 3 of the three stage process. Maintenance of erythorbate was calculated based on measured erythorbate concentration and tank volume.

The conditions for the two-stage process were as follows: Stage one tank conditions were 400 gallons at 0.43% sodium bicarbonate, 0.18% sodium hydroxide, pH 10.4, 24.2° C., 23 sec dwell time. Stage two tank conditions were 550 gallons at 2.4% sodium erythorbate, 0.6% erythorbic acid, 0.1% calcium chloride, 0.1% EDTA, pH 4.7, 15.8° C., 60 sec dwell time.

The conditions for the three-stage process were as follows: Stage one tank conditions were 400 gallons at 0.43% sodium carbonate, 0.084% sodium bicarbonate, pH 10.4, 21.2° C., 30 sec dwell time. Stage two tank conditions were 550 gallons at 0.49% citric acid, 0.72% sodium citrate, pH 4.5, 18° C., 35 sec dwell time. Stage three tank conditions were 150 gallons at 3% sodium erythorbate, 0.1% calcium chloride, 0.1% EDTA, 15 sec dwell time.

The depletion of erythorbate is two to three times greater for the two-stage process compared to the three-stage process. Approximately three times the weight of mushrooms can be processed per LB of sodium erythorbate using the three-stage process compared to the two-stage process.

Two-Stage Process

Mushrooms Processed (lbs)	Measured Tank 2 (550 gal) Erythorbate Concentration (%)	Weight erythorbate added (sodium erythorbate plus erythorbic acid) (lbs)
100	3.0	137.6 initial
1700	2.3	
3070	2.9	
9000	2.2	
12070	1.9	

Calculation of erythorbate used: 137.6 − [(1.9%)(1,900)] = 98.5 lbs.
Calculation of erythorbate used per lb mushroom processed: 98.5 lbs/12070 lbs = 0.0082 lbs erythorbate per lb mushroom processed.
LBS of mushrooms/LB of sodium erythorbate: 12,070/98.5 = 122.5

Three-Stage Process

Mushrooms Processed (lbs)	Measured Tank 3 (150 gal) Erythorbate Concentration (%)	Weight sodium erythorbate added (lbs) (to keep concentration ≈ 3%)
0	2.9	47.3 initial
1229	2.7	
2400	2.6	
3144	2.3	
3939	2.1	
5246	3.1	15.6
6973	2.9	
7801	2.5	
9048	2.4	
10379	2.3	
13438	2.0	

Calculation of erythorbate used: 62.9 − [(47.3)(2.0/2.9%)] = 28.4
Calculation of erythorbate used per lb mushroom processed: 28.4 lbs/13438 lbs = 0.0021 lbs erythorbate per lb mushroom processed.
LBS of mushrooms processed per LB of sodium erythorbate: 13438/28.4 = 473.2 LBS

EXAMPLE 9

Two-Stage vs. Three-Stage Process: Effect on Cost of Charging Commercial Scale Mushroom Wash Line

The costs of filling commercial sized tanks for the two-stage process were compared to the three stage process. Costs are represented as indexed costs with the cost of calcium disodium EDTA arbitrarily set at 100. Cost of the other individual ingredients is relative to EDTA.

The conditions for the two-stage process were as follows: Stage one tank conditions were 400 gallons at 0.43% sodium bicarbonate, 0.18% sodium hydroxide, pH 10.4, 24.2° C., 23 sec dwell time. Stage two tank conditions were 550 gallons at 2.4% sodium erythorbate, 0.6% erythorbic acid, 0.1% calcium chloride, 0.1% EDTA, pH 4.7, 15.8° C., 60 sec dwell time.

The conditions for the three-stage process were as follows: Stage one tank conditions were 400 gallons at 0.43% sodium carbonate, 0.084% sodium bicarbonate, pH 10.4, 21.2° C., 30 sec dwell time. Stage two tank conditions were 550 gallons at 0.49% citric acid, 0.72% sodium citrate, pH 4.5, 18° C., 35 sec dwell time. Stage three tank conditions were 150 gallons at 3% sodium erythorbate, 0.1% calcium chloride, 0.1% EDTA, 15 sec dwell time.

The addition of a third treatment tank, and separation of the neutralization and browning inhibitor treatments as practiced in the three-stage process, results in significantly lower costs to charge commercial size treatment tanks as shown in the analysis below. The initial cost to fill the three tanks in the three-stage process is 60% less than the cost to fill the two tanks in the two-stage process.

Two-Stage Process

Ingredient	Initial Charge (lbs)	Indexed Ingredient Cost (ICIC)[1]	Indexed Cost Initial Charge (ICI)[2]
Stage One Tank 400 gallons			
sodium	14.14	16.07	228.54
bicarbonate			
Total			228.54

[1]Indexed Ingredient Cost (ICIC): The actual 1998 ingredient costs per pound were divided by the actual 1998 cost of calcium disodium EDTA per pound and multiplied by 100.
[2]Indexed Cost Initial Charge (ICI): (ICIC) × Initial Charge (Lbs)

Ingredient	Initial Charge (lbs)	Indexed Ingredient Cost (ICIC)[1]	Indexed Cost Initial Charge (ICI)[2]
Stage Two Tank 550 gallons			
sodium erythorbate	110.1	81.34	8953.42
erythorbic acid	27.5	148.65	3860.89
calcium disodium EDTA	4.8	100	460.00
calcium chloride	4.8	11.43	98.57
Total			11300.97

[1]Indexed Ingredient Cost (ICIC): The actual 1998 ingredient costs per pound were divided by the actual 1998 cost of calcium disodium EDTA per pound and multiplied by 100.
[2]Indexed Cost Initial Charge (ICI): (ICIC) × Initial Charge (lbs)

Total indexed cost for charging tanks for two-stage process: 11319.51

Three-Stage Process

Ingredient	Initial Charge (lbs)	Indexed Ingredient Cost (ICIC)[1]	Indexed Cost Initial Charge (ICI)[2]
Stage One Tank 400 gallons			
sodium carbonate	14.14	38.87	120.51
sodium	1.60	37.72	28.14
bicarbonate			
Total			208.65

[1]Indexed Ingredient Cost (ICIC): The actual 1998 ingredient costs per pound were divided by the actual 1998 cost of calcium disodium EDTA per pound and multiplied by 100.
[2]Indexed Cost Initial Charge (ICI): (ICIC) × Initial Charge (lbs)

Ingredient	Initial Charge (lbs)	Indexed Ingredient Cost (ICIC)[1]	Indexed Cost Initial Charge (ICI)[2]
Stage Two Tank 550 gallons			
citric acid	22.45	17.35	389.44
sodium	33.94	25.82	877.10
citrate			
Total			1245.78

[1]Indexed Ingredient Cost (ICIC): The actual 1998 ingredient costs per pound were divided by the actual 1998 cost of calcium disodium EDTA per pound and multiplied by 100.
[2]Indexed Cost Initial Charge (ICI): (ICIC) × Initial Charge (lbs)

Ingredient	Initial Charge (lbs)	Indexed Ingredient Cost (ICIC)[1]	Indexed Cost Initial Charge (ICI)[2]
Stage Three Tank 150 gallons			
sodium erythorbate	47.50	68.34	3851.4
calcium chloride	1.4	11.43	14.32
calcium disodium EDTA	1.3	100	130.00
Total			3031.72

[1]Indexed Ingredient Cost (ICIC): The actual 1998 ingredient costs per pound were divided by the actual 1998 cost of calcium disodium EDTA per pound and multiplied by 100.
[2]Indexed Cost Initial Charge (ICI): (ICIC) × Initial Charge (lbs)

Total indexed cost for charging tanks: 4565.34

EXAMPLE 10

Reduction of microbial populations on mushrooms washed by the three-stage process.

Mushrooms were washed in a commercial trial with the three-stage process. The conditions for the three-stage process were as follows: Stage one tank conditions were 0.43% sodium carbonate, 0.084% sodium bicarbonate, pH 11.1, 25.0° C., 30 sec dwell time. Stage two tank conditions were 0.49% citric acid, 0.72% sodium citrate, pH 4.5, 15 sec dwell time. Stage three tank conditions were 3% sodium erythorbate, 0.2% calcium chloride, 0.1% EDTA, 35 sec dwell time.

Triplicate samples were collected at each sampling point. All samples were analyzed for aerobics and fluorescent pseudomonads.

As shown in FIG. 1, the three-stage process resulted in an average aerobic population reduction of 17-fold in the washed mushrooms. An 11-fold reduction was found in pseudomonas population as reflected in FIG. 2.

We claim:

1. A method for preserving mushrooms comprising the steps of:

contacting the mushrooms with an aqueous anti-microbial solution having a pH of from about 10.5 to about 11.5;

rinsing the mushrooms at least once with at least one aqueous pH neutralizing buffer solution comprising organic acid and at least one salt of an organic acid and substantially free from erythorbic acid and sodium erythorbate; and

contacting the mushrooms at least once with at least one solution comprising a browning inhibitor and a chelating agent.

2. A method of claim 1 wherein the anti-microbial solution comprises at least one of sodium bicarbonate, sodium carbonate and sodium hydroxide.

3. A method of claim 2 wherein the anti-microbial solution comprises from about 0.3% to about 0.5% by weight sodium carbonate and from about 0.05% to about 0.10% by weight sodium bicarbonate.

4. A method of claim 3 wherein the contacting with an anti-microbial solution is carried out for from about 20 to about 40 seconds at about 25° C.

5. A method of claim 1 wherein the browning inhibitor is selected from the group consisting of sodium erythorbate, erythorbic acid, ascorbic acid, calcium ascorbate and L-cysteine.

6. A method of claim 1 wherein the organic acid and salt of an organic acid comprise citric acid and sodium citrate, respectively.

7. A method of claim 6 wherein the rinsing step is carried out with a pH of about from 3.0 to 5.0 for about from 10 to 30 seconds.

8. A method of claim 7 wherein the browning inhibitor and chelating agent comprise sodium erythorbate and calcium disodium EDTA, respectively.

9. A method of claim 8 wherein the solution used in the anti-browning treating step further comprises calcium chloride and the contacting is carried out for about 20 to about 40 seconds.

10. A method of claim 9 wherein the solution in at least one step further includes an antifoaming or defoaming solution.

11. A method of claim 10 wherein the antifoaming solution comprises at least one silicon emulsion defoamer or antifoam.

12. A method of claim 6 wherein the solution used in the treating step further comprises calcium chloride.

13. A method of claim 12 wherein the solution in at least one treating step further includes an antifoaming or defoaming solution.

14. A method of claim 6 wherein the contacting is carried out for about 20 to about 40 seconds.

15. A method of claim 14 wherein the antifoaming solution comprises at least one silicon emulsion defoamer or antifoam.

16. A method of claim 15 wherein said organic acid comprises about from 0.4% to about 0.6% by weight citric acid and said salt of said organic acid comprises from about 0.6% to about 0.8% by weight sodium citrate with a pH of about from 3.0 to 5.0.

17. A method of claim 16 wherein said browning inhibitor is at least one selected from the group consisting of sodium erythorbate, erythorbic acid, ascorbic acid, calcium ascorbate and L-cysteine hydrochloride.

18. A method of claim 13 wherein the anti-browning step solution has a pH from about 6.5 to about 7.5.

19. A method of claim 18 wherein the antifoaming solution comprises at least one silicon emulsion defoamer or antifoam.

20. A method of claim 19 wherein the anti-microbial solution comprises from about 0.3% to about 0.5% by weight sodium carbonate and from about 0.05% to about 0.10% by weight sodium bicarbonate.

21. A method of claim 20 wherein the solution in at least one contacting step further includes an antifoaming or defoaming solution.

22. A method of claim 15 wherein the anti-microbial solution comprises at least one of sodium bicarbonate, sodium carbonate and sodium hydroxide.

23. A method of claim 22 wherein the contacting with an anti-microbial is carried out for about from 20 to about 40 seconds at a temperature of about 25° C.

24. A method of claim 23 wherein the antifoaming solution comprises at least one silicon emulsion defoamer or antifoam.

25. A method of claim 24 wherein the browning inhibitor and chelating agent comprise sodium erythorbate and calcium disodium EDTA, respectively, with a pH from about 6.8 to about 7.5.

26. A method of claim 25 wherein the antifoaming solution comprises at least one silicon emulsion defoamer or antifoam.

27. A method for preserving mushrooms comprising the steps of:

contacting the mushrooms with an anti-microbial solution having a pH from about 10.5 to about 11.5;

rinsing the mushrooms at least once with at least one aqueous pH neutralizing buffer solution comprising citric acid and sodium citrate and substantially free from erythorbic acid and sodium erythorbate; and

treating the mushrooms at least once with at least one solution comprising a browning inhibitor and a chelating agent.

28. A method of claim 27 wherein said rinsing is carried out with a pH of about from 4.0 to 4.5 for about from 10 to 20 seconds.

29. A method of claim 28 wherein said chelating agent comprises calcium disodium EDTA.

30. A method of claim 27 wherein the solution in at least one step further includes an antifoaming or defoaming solution.

31. A method for preserving mushrooms comprising the steps of:

contacting the mushrooms with an anti-microbial solution comprising sodium bicarbonate, sodium carbonate and sodium hydroxide, the solution having a pH from about 10.5 to about 11.5;

rinsing the mushrooms at least once with at least one aqueous pH neutralizing buffer solution of at least one organic acid and at least one salt of an organic acid and substantially free from erythorbic acid and sodium erythorbate; and

treating the mushrooms at least once with at least one solution comprising a browning inhibitor and a chelating agent with a pH from about 6.5 to about 7.5 and a divalent cation.

32. A method of claim 31 wherein the solution in at least one step further includes an antifoaming or defoaming solution.

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EXHIBIT B

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RE: U.S. Patent No. 5,919,507 (Issued July 6, 1999) and PCT/US98/20728 (Filed October 2, 1998)
"Novel Two-Stage Wash Process for Extending Shelf Life of Fresh Mushrooms"
Inventors: R. Beelman, *et al.*
PSU Invention Disclosure No. 95-1514

Dear

We received your letter dated / and your inquiry about the University's other technology rights in the area of mushroom treatment.

As you know, PSRF is the owner of US Patent 5,919,507. This patent covers, inter alia, a preferred embodiment of the University's process. However, we believe that the claims drafted by EPL's counsel were not as broad as the invention described and enabled in the patent specification. Accordingly, the University has filed documents at the U.S. Patent and Trademark Office to expand the breadth of the patent.

PSRF has also filed PCT international application PCT/US98/20728 corresponding to the '507 patent. PSRF has entered into the national phase of numerous countries outside of the US based on this application.

The University has also filed a new application covering refinements and improvements to the initially-developed process, including a process different from the two-stage wash claimed in the '507 patent. The resulting patent is expected to cover the results of further research and development conducted by Dr. Beelman's laboratory since the original application was filed.

If is interested in licensing these technologies for either domestic use or international use, we would be willing to provide additional information about the University's latest processes and proprietary rights.

Sincerely,

Matthew Smith
Technology Licensing Officer

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